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EXPERIMENTAL STUDY OF CHEMOTHERAPY RELATED LEUKOCYTOPENIA TREATED BY VARIOUS PEROAL LEUCOCYTE INCREASING DRUGS

Zhang Xiping[1]，Yang Hongjian[1*]，Zou Dehong[1]，He Xiangming[1]，Yu Xingfei[1]，Li Yongfeng[1]

Department of Breast Surgery, Zhejiang Cancer Hospital, Hangzhou 310022, Zhejiang Province, China

***Correspondence to:** Yang Hongjian, Department of Breast Surgery, Zhejiang Cancer Hospital, Banshanqiao, No.38 Guangji Road, Hangzhou 310022, Zhejiang Province, China
E-mail: 626876448@qq.com

Abstract

Background: Clinically, the patients with significant WBC decrease are mostly administered G-CSF, this kind of drugs is expensive and adverse reactions are often seen. In contrast, oral leucocyte increasing drug has small adverse reactions, can be used for longer time and can improve the continuity and stability of treatment. The experimental study based on study of mouse was to evaluate the effects of treatment and chemotherapy of related leukocytopenia by five kinds of commonly used peroal leucocyte increasing drugs.

Materials and Methods: We prepared mice chemotherapy related leukocytopenia model by cyclophosphamide intraperitoneal injection, the positive control drug is G-CSF, respectively fill five kinds of peroal Leucocyte increasing drugs (Qijiao Shengbai Capsule, Weixuening Granule, Compound Zaofan Pill, Berbamine and Leucogen Tablets) in the stomach, the experimental group was divided into normal control group (group A), model group (group B), positive control group (Group rhG-CSF, group C) and treatment groups (group D-H), and treatment groups were divided into Qijiao Shengbai Capsule group (group D), Weixuening Granule group (group E), Compound Zaofan Pill group (group F), Berbamine Tablet group (group G) and Leucogen Tablet group (group H). Calculate the death rate, blood routine and important visceral organ index in each group..

Results: The death rate of mice in each group has no significant difference (P>0.05). WBC of B, D, E and F groups was significantly lower than that of group A (P<0.05 or P<0.01). WBC of C, G and H groups was significantly higher than those of group B (P<0.01). WBC of D, E and F groups was significantly lower than that of group C (P<0.01). WBC of G and H groups was significantly higher than that of D and F groups (P<0.01), WBC of group H is significantly higher than that of group E (P<0.05). RBC of group F, G and H groups was significantly higher than that of group D (P<0.05 or P<0.01). HB of group H is significantly higher than that of group A (P<0.01). HB of C, G and H groups was significantly higher than that of group B (P average <0.01). HB of D, E and F groups was significantly lower than that of group C (P<0.05 or P<0.01). HB of G and H groups was significantly higher than that of D, E and F groups (P average <0.01). PLT of group H was significantly higher than that of group B (P average <0.05). PLT of F, G and H groups was significantly higher than that of group D (P<0.01). Lung index of group G was significantly higher than that of D, E, F and H groups (P<0.01). Liver index of group H is significantly higher than that of group D (P<0.05). Thymus index of G and H groups is significantly higher than that of group F (P<0.05 or P<0.01).

Conclusions: Among all drugs of rising WBC, G-CSF owns strongest effect. In oral drug groups, WBC rising effect of Leucogen Tablets is best, RBC, HB and PLT improvement effect of Berbamine and Leucogen Tablets is best. In addition, Berbamine and Leucogen Tablets respectively caused significant increase of lung and liver index, what indicates that, the two drugs may be accompanied by relevant viscera damage. At the same time, the two drugs also

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increased thymus index, which indirectly indicates that, the immunity and regulation abilities of Berbamine and Leucogen Tablets are stronger. The spleen index of Qijiao Shengbai Capsule group was significantly higher than that of Berbamine Tablet and Leucogen Tablet groups, what indicates that, the immunity and regulation abilities of Qijiao Shengbai Capsule may be stronger in oral drug group.

Keywords: leucocyte increasing drugs; chemotherapy; leukocytopenia; mouse

Introduction

At present, chemotherapy is one of the important methods in treating malignant tumors, myelosuppression is the most important blood toxicity, in which, leucopenia after chemotherapy is the most common phenomenon of myelosuppression, is also one of main reasons of easy concurrent infection. Clinically, WBC $<4*10^9$/L is called leucopenia and neutrophil $<2*10^9$/L is called neutropenia, sometimes can be associated with chill, fever and more prone to serious infections to cause sepsis or toxic hepatitis, the mouth, nose, skin, rectum and anal mucosa may occur ulceration, what seriously affects normal treatment of tumor patients, even threatens their life, so should carry out close monitoring and gives timely treatment (Li et al.,2010). At present, in terms of III-IV degree WBC decrease, generally is treated by granulocyte colony stimulating factor (G-CSF) in clinic, and I - II degree decrease is treated by peroal Leucocyte increasing drug. In clinic, there are many peroal Leucocyte increasing drugs, but these drugs have no repoted systematic evaluation and comparison to treatment value in leucopenia caused by chemotherapy, so there may be some confusion in the use. This paper evaluates the efficacy of commonly used peroal Leucocyte increasing drugs in clinic through animal experiments in mice, and hopes to provide a reference for clinical applications.

Materials and Methods

Experimental materials

Experimental animals and groups

Laboratory Animal Center, School of Medicine, Xi'an Jiao Tong University (Xi'an, China) provides 132 clean-grade Kunming male mice [License No.: SCXK (Shaanxi) 2007-001], with average weight of 18-22g, the experimental groups are divided into control group (Group A), model group (Group B), positive control group (group rhG-CSF, Group C) and treatment groups (Group D-H), the treatment groups are divided into Qijiao Shengbai Capsule (Group D), Wei Xue Ning granule group (Group E), Compound Zaofan Pill group (Group F), Berbamine group (Group G) and Leucogen group (Group H). Normal control group, model group and positive control group (group rhG-CSF) respectively has 24 mice, and the rest of treatment group owns 12 mice.

Experimental drugs

Berbamine tablet (main component is berbamine, produced by Sichuan Jinshan Chanxin Pharmaceutical Co., Ltd, MPN: H51023699), Compound Zaofan Pill (produced by Shaanxi Hao Qijun Pharmaceutical Co., Ltd, MPN: Z61020457), Qijiao Shengbai Capsule (produced by Guiyang Dechangxiang Pharmaceutical Co., Ltd, MPN: Z20025027), Wei Xue Ning (produced by Pharmaceutical Factory of Shaanxi College of Traditional Chinese Medicine, MPN: Z20044038), Leucogen (produced by Jiangsu Gibel Pharmaceutical Co., Ltd, MPN: H32025444), rhG-CSF (recombinant human granulocyte colony-stimulating factor injection, produced by Qilu Pharmaceutical Co., Ltd, MPN: S20063065), cyclophosphamide (produced by Shanxi Pude Pharmaceutical Co., Ltd, MPN: H14023686).

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Main instruments

MB-1830 whole blood cell analyzer (Wuhan Zhuo'er Medical Equipment Co., Ltd, China), electronic balance (Shanghai Fangrui Instrument Co., Ltd, China).

Experimental methods

Preparation of chemotherapy related leukocytopenia model

After normal feeding of the mice for 3 days, we injected cyclophosphamide (CTX) 100mg/kg in abdominal cavity of mice, once daily and continuously for 3 days, using 1 mL sterile syringe after treated by heparin to draw peripheral blood 50-100μL through inner canthus of mice before CTX injection and on 2nd, 3rd, 4th and 5th day after injecting CTX, then count total WBC of mice under the microscope. Mice model will be successfully established if WBC number is decreased gradually, finally stabilized at a low level and there is significant statistical difference compared with what before injection(Zhang et al., 2008; Li et al., 2009; Huang et al., 2010).

Experimental animals' administration mode, dosage and concentration

Each group was first administered for 3 days according to the calculated dosage and once daily. B-H groups were injected CTX 100mg/kg/d in abdominal cavity from the 4th day, group A was injected the same amount of normal saline in abdominal cavity, once daily and continuously 3 days. The dosage is calculated according to direct conversion method (Xu et al., 2002) of equivalent dosage among different animals of Xu Shuyun's *Pharmacological Experimental Methodology* (Third Edition, China), the dosage of mice is equal to 9 times of normal adults in clinic. A-B groups are filled stomach with normal saline, group C was injected subcutaneously rhG-CSF 10μg/kg, group D is filled stomach with Qijiao Shengbai Capsule 2.0g/kg, group E is filled stomach with Wei Xue Ning 30mL/kg, group F is filled stomach with Compound Zaofan Pill 2.0g/kg, group G is filled stomach with berbamine 56mg/kg, and group H is filled stomach with Leucogen 10mg/kg, all were continuous for 9 days. Administration was three days before modeling, once daily and for a total of nine days.

Detection of relevant indexes

Weigh on 3rd, 6th and 9th day of modeling. On 9th day of modeling, namely, 6th day after injecting cyclophosphamide, blood samples were drawn from each mouse from posterior orbital venous plexus to measure the number of WBC, RBC and platelet, as well as hemoglobin content. Kill the mice on the 12th day of modeling. We weighed the mice, dissected vital organs and weighed it, calculate organ index according to the weight of death and analyze the effect of different oral Leucocyte increasing drugs to mice vital organs, endocrine gland and gonad index. The organ index = organ weight / animal body weight.

Statistical analysis

SPSS 19.0 was adopted for statistical analysis of the data, and the measurement was expressed by $\overline{x} \pm s$, the groups comparison using single factor variance analysis, the comparison between the two is tested by *LSD-T*, the death rate comparison uses Fisher direct probability method, $P<0.05$ means that there is statistical difference and $P<0.01$ means that the difference was significant.

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Results

Comparison of death rate of each group mice (Table 1):

Table 1: Comparison of death rate of each group mice

Group	Number (n)	Death number (n)	Death rate (%)
A	24	2	8.33
B	24	4	16.67
C	24	5	20.83
D	12	2	16.67
E	12	3	25.00
F	12	0	0
G	12	0	0
H	12	3	25.00

Note: Compared with group B, the death rate of each group has no significant difference (P>0.05).

The reason for the number of death recorded in group E and H mice was due to stomach filling; while that of group B and D may be that WBC was too low; hence resistance was low; the cause for group A and C is not clear; the mice in group F and G were alive. Compared with group B, the death rate of each group has no significant difference (P>0.05).

Influence of different oral Leucocyte increasing drugs to blood three lines of mice (Table 2)

Comparison of changes of WBC: (1) Compared with group A: group B, D, E and F are significantly lower than that of group A (P<0.05 or P<0.01). (2) Compared with group B: group C, G and H are significantly higher than that of group B (P<0.01). (3) Compared with group C: group D, E and F are significantly lower than that of group C (P<0.01). (4) Comparison among other groups: group G and H are significantly higher than what of group D and F (P<0.01), group H is significantly higher than what of group E (P<0.05).

Table 2: Influence of different oral Leucocyte increasing drugs to blood three lines of mice

Group	N	WBC（10^9/L）	LY%（%）	NE%（%）	RBC（10^{12}/L）	Hb（g/L）	PLT（10^9/L）
A	22	5.73 ± 1.89	71.36 ± 8.60	25.22 ± 8.35	8.27 ± 0.69	129.79 ± 31.39	495.55 ± 99.20
B	20	2.60 ± 1.53**	71.60 ± 9.30	24.62 ± 8.57	7.55 ± 0.35**	109.39 ± 19.10**	413.40 ± 117.17*
C	19	6.95 ± 3.34$^{\Delta\Delta}$	59.24 ± 13.19**$^{\Delta\Delta}$	34.69 ± 11.98**$^{\Delta\Delta}$	7.88 ± 0.69	138.81 ± 18.14$^{\Delta\Delta}$	402.74 ± 119.86*
D	10	3.23 ± 1.42**$^{\blacktriangle\blacktriangle}$	64.31 ± 9.61	29.76 ± 9.84	7.51 ± 0.98**	114.65 ± 22.58$^{\blacktriangle\blacktriangle}$	315.10 ± 85.55**$^{\Delta}$
E	9	4.03 ± 1.81*$^{\blacktriangle\blacktriangle}$	60.39 ± 17.51*$^{\Delta}$	31.77 ± 18.06	7.90 ± 1.01	110.82 ± 26.73*$^{\blacktriangle\blacktriangle}$	404.89 ± 66.43
F	12	3.04 ± 0.81**$^{\blacktriangle\blacktriangle}$	67.23 ± 14.43	27.40 ± 13.35	8.19 ± 0.47$^{\Delta}$	116.93 ± 12.84$^{\blacktriangle}$	467.58 ± 144.83
G	12	5.89 ± 2.95$^{\Delta\Delta}$	73.24 ± 10.77$^{\blacktriangle\blacktriangle}$	22.12 ± 9.14$^{\blacktriangle\blacktriangle}$	8.31 ± 0.59$^{\Delta\Delta}$	145.84 ± 21.98$^{\Delta\Delta}$	495.23 ± 65.71$^{\blacktriangle}$
H	9	6.60 ± 1.58$^{\Delta\Delta}$	74.47 ± 14.50$^{\blacktriangle\blacktriangle}$	23.63 ± 13.36$^{\blacktriangle}$	8.35 ± 0.74$^{\Delta\Delta}$	153.43 ± 17.23**$^{\Delta\Delta}$	510.33 ± 232.92$^{\Delta\blacktriangle}$

Note: Compared with group A,*P<0.05, **P<0.01; Compared with group B, $^{\Delta}P$<0.05, $^{\Delta\Delta}P$<0.01; Compared with group C, $^{\blacktriangle}P$<0.05，$^{\blacktriangle\blacktriangle}P$<0.01

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Comparison of changes of LY%: (1) Compared with group A and B: group C and E are significantly lower than what of group A (P<0.05 or P<0.01). (2) Compared with group C: group G and H are significantly higher than what of group C (P<0.01). (3) Comparison among other groups: group G and H are significantly higher than that of group E (P<0.05).

Comparison of changes of NE %: (1) Compared with group A: group C is significantly higher than thatof group A (P<0.01). (2) Compared with group B: group C is significantly higher than that of group B (P<0.01). (3) Compared with group C: group G and H are significantly lower than that of group C (P<0.05 or P<0.01). (4) Comparison among other groups: there is no significant difference (P>0.05).

Comparison of changes of RBC: (1) Compared with group A: group B and D are significantly lower than that of group A (P<0.01). (2) Compared with group B: group F, G and H are significantly higher than that of group B (P<0.05 or P<0.01). (3) Compared with group C: there was no significant difference (P>0.05). (4) Comparison among other groups: group F, G and H are significantly higher than that of group D (P<0.05 or P<0.01).

Comparison of changes of HB: (1) Compared with group A: group B and E are significantly lower than that of group A (P<0.05 or P<0.01), group H was significantly higher than that of group A (P<0.01). (2) Compared with group B: group C, G and H are significantly higher than what of group B (P<0.01). (3) Compared with group C: group D, E and F are significantly lower than that of group C (P<0.05 or P<0.01). (4) Comparison among other groups: group G and H are significantly higher than that of group D, E and F (P<0.01).

Comparison of changes of PLT: (1) Compared with group A: group B, C and D are significantly lower than that of group A (P<0.05 or P<0.01). (2) Compared with group B: group D is significantly lower than that of group B, group H was significantly higher than that of group B (P<0.05). (3) Compared with group C: group G and H are significantly higher than that of group C (P<0.05). (4) Comparison among other groups: group F, G and H are significantly higher than that of group D (P<0.01).

Table 3: Effects of different drugs to mice vital organs（Unit: mg/10g）

Group	N	Heart	Liver	Spleen	Lung	kidney
	22	55.55 ± 8.77	621.65 ± 51.37	53.52 ± 18.81	73.76 ± 13.50	168.83 ± 12.71
B	20	62.51 ± 16.08*	660.42 ± 114.60	65.48 ± 33.95	114.44 ± 29.08**	164.43 ± 38.04
C	19	55.86 ± 7.34$^{\Delta}$	618.89 ± 66.56	110.66 ± 69.02**$^{\Delta\Delta}$	100.07 ± 28.88**	153.83 ± 16.82*
D	10	46.98 ± 10.04*$^{\Delta\Delta\blacktriangle}$	565.44 ± 128.11$^{\Delta\Delta}$	99.04 ± 21.30**$^{\Delta}$	93.06 ± 10.99*	148.56 ± 14.87$^{\Delta}$
E	9	48.45 ± 8.67$^{\Delta\Delta}$	606.09 ± 45.88	92.33 ± 23.04*	84.41 ± 20.81	144.71 ± 13.70**
F	12	46.49 ± 5.41*$^{\Delta\Delta\blacktriangle}$	581.49 ± 42.85$^{\Delta\Delta}$	96.94 ± 38.33**$^{\Delta}$	85.56 ± 8.75	143.24 ± 9.78**$^{\Delta\Delta}$
G	12	53.65 ± 8.27$^{\Delta}$	613.63 ± 48.51	66.59 ± 29.03$^{\blacktriangle\blacktriangle}$	112.71 ± 27.03**	155.91 ± 8.95
H	9	63.04 ± 11.24	638.71 ± 75.34	38.97 ± 9.51$^{\blacktriangle\blacktriangle}$	87.50 ± 18.99	163.18 ± 14.09$^{\blacktriangle}$

Note: Compared with group A,*P<0.05, **P<0.01; Compared with group B, $^{\Delta}P$<0.05, $^{\Delta\Delta}P$<0.01; Compared with group C, $^{\blacktriangle}P$<0.05，$^{\blacktriangle\blacktriangle}P$<0.01

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Table 4: Effects of different drugs to endocrine gland and gonad index of mice（Unit：mg/10g）

Group	N	Thymus	Adrenal gland	Testis	Seminal vesicle prostate	Foreskin gland
A	22	20.40 ± 6.46	4.11 ± 1.50	67.52 ± 13.66	62.03 ± 13.66	27.67 ± 6.98
B	20	9.71 ± 5.41**	2.94 ± 1.57**	81.11 ± 14.98*	48.85 ± 25.60*	28.09 ± 24.77
C	19	10.71 ± 3.86**	2.20 ± 0.66**	88.36 ± 29.03**	50.00 ± 17.99*	22.65 ± 10.83
D	10	7.84 ± 4.39**	2.55 ± 1.39**	92.80 ± 7.82**	37.10 ± 9.08**	15.82 ± 5.59*$^{\Delta}$
E	9	9.74 ± 3.03**	2.08 ± 1.58**	97.45 ± 14.92**$^{\Delta}$	43.32 ± 20.29**	17.99 ± 7.96
F	12	5.72 ± 1.00**$^{\Delta}$▲	1.74 ± 0.64**$^{\Delta}$	88.03 ± 8.27**	33.60 ± 9.97**$^{\Delta}$▲	12.72 ± 4.43**$^{\Delta\Delta}$▲
G	12	11.91 ± 7.67**	2.92 ± 1.38*	102.42 ± 20.22**$^{\Delta\Delta}$▲	38.06 ± 15.26**	19.23 ± 14.65
H	9	12.03 ± 7.72**	2.84 ± 0.55*	77.76 ± 18.85	52.25 ± 14.64	37.22 ± 6.95$^{▲▲}$

Note： Compared with group A,*P<0.05, **P<0.01; Compared with group B, $^{\Delta}P$<0.05, $^{\Delta\Delta}P$<0.01; Compared with group C, $^{▲}P$<0.05，$^{▲▲}P$<0.01

Comparison of changes of cardiac index: (1) Compared with group A: group B was significantly higher than that of group A, group D and F are significantly lower than that of group A (P<0.05). (2) Compared with group B: group C, D, E, F and G are significantly lower than that of group B (P<0.05 or P<0.01). (3) Compared with group C: group D and F are significantly lower than that of group C (P<0.05). (4) Comparison among other groups: group H was significantly higher than that of group D, E, F and G (P<0.05 or P<0.01).

Comparison of changes of liver index: (1) Compared with group B: group D and F are significantly lower than that of group B (P<0.01). (2) Compared with group A and C: there was no significant difference (P>0.05). (3) Comparison among other groups: group H was significantly higher than that of group D (P<0.05).

Comparison of changes of spleen index: (1) Compared with group A: group C, D, E and F are significantly higher than that of group A (P<0.05 or P<0.01). (2) Compared with group B: group C, D and F are significantly higher than that of group B (P<0.05 or P<0.01). (3) Compared with group C: group G and H are significantly lower than that of group C (P<0.01). (4) Comparison among other groups: group G and H are significantly lower than that of group D (P<0.05 or P<0.01), group H was significantly lower than that of group E and F (P<0.01).

Comparison of changes of lung index: (1) Compared with group A: group B, C, D and G are significantly higher than what of group A (P<0.05 or P<0.01). (2) Compared with group B: group C, D, E, F and H are significantly lower than what of group B (P<0.05 or P<0.01). (3) Compared with group C: there is no significant difference (P>0.05). (4) Comparison among other groups: group G is significantly higher than what of group D, E, F and H (P<0.01).

Comparison of changes of renal index: (1) Compared with group A: group C, E and F are significantly lower than that of group A (P<0.05 or P<0.01). (2) Compared with group B: group D and F are significantly lower than that of group B (P<0.05 or P<0.01). (31) Compared with group C: there was no significant difference (P>0.05). (4)

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Comparison among other groups: group H was significantly higher than that of group F (P<0.05).

Comparison of changes of thymus index: (1) Compared with group A: the groups are significantly lower than what of group A (P<0.01). (2) Compared with group B: group F is significantly lower than what of group B (P<0.05). (3) Compared with group C: group F is significantly lower than what of group C (P<0.05). (4) Comparison among other groups: group G and H are significantly higher than that of group F (P<0.05 or P<0.01).

Comparison of changes of adrenal index: (1) Compared with group A: group B, C, D, E, F, G and H are significantly lower than that of group A (P<0.05 or P<0.01). (2) Compared with group B: group F is significantly lower than that of group B (P<0.05). (3) Compared with group C: there was no significant difference (P>0.05). (4) Comparison among other groups: group G is significantly higher than that of group F (P<0.05).

Comparison of changes of testis index: (1) Compared with group A: group B, C, D, E, F and G are significantly higher than that of group A (P<0.05 or P<0.01). (2) Compared with group B: group E and G are significantly higher than that of group B (P<0.05 or P<0.01). (3) Compared with group C: group G was significantly higher than that of group C (P<0.05). (4) Comparison among other groups: group E and G are significantly higher than that of group H (P<0.05 or P<0.01).

Comparison of changes of seminal vesicle prostate index: (1) Compared with group A: group B, C, D, E, F and G are significantly lower than that of group A (P<0.05 or P<0.01). (2) Compared with group B: group F was significantly lower than that of group B (P<0.05). (3) Compared with group C: group F was significantly lower than that of group C (P<0.05). (4) Comparison among other groups: group H was significantly higher than that of group F (P<0.05).

Comparison of changes of preputial gland index: (1) Compared with group A: group D and F are significantly lower than that of group A (P<0.05 or P<0.01). (2) Compared with group B: group D and F are significantly lower than that of group B (P<0.05 or P<0.01). (3) Compared with group C: group F was significantly lower than that of group C, group H is significantly lower than that of group C (P<0.01). (4) Comparison among other groups: group D, E, F and G are significantly lower than that of group H (P<0.01).

Discussion

At present, there are many drugs that increase WBC; the commonly used include: Berbamine tablet, Qijiao Shengbai Capsule, Shengxuebao mixture, WBC increasing mixture, Leucogen tablet, Compound Zaofan Pill and Wei Xue Ning, etc, also, G-CSF and GM-CSF, etc. Those several drugs are those commonly used in our hospital for the leukocytopenia patients. The mechanism of different drugs improving the white blood cell is also different.

Qijiao Shengbai Capsule originated from ethnic Hmong folk prescription, it selects precious medicinal materials and uses modern scientific methods to refine again and again. Qijiao Shengbai Capsule not only can tonifying qi and blood, improved the immunity of the human body, but also can rapidly increase the proliferation of white blood cells. Weixuening Granule can improve blood circulation and supply. It can nourish the liver and kidney, cleanse the blood and reduce body temperature. It is used in the reduction of platelets and white blood cells. The effective ingredient of Berbamine tablet is berbamine, which can stimulate pulp cell proliferation, improve the content of hematopoietic stem cell colony factor, and increase proliferation of hematopoietic stem cells and granule progenitor cells. It can be used to

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treat different leukocytopenia. Compound Zaofan Pills can protect the kidney and marrow, supplementing Qi and nourishing Yin. It can be used in aplastic anemia, neutropenia, thrombocytopenia, bone marrow hyperplasia, and bone marrow damage induced by radiation and chemotherapy. Leukogen is 2-(2-Ethoxy-2-oxo-1-phenylethyl)-1,3-thiazolidine-4-carboxylic acid; 2-thiazolidineacetic acid, 4-carboxy-alpha-phenyl-, alpha-ethyl ester. It can be used to prevent and treat various eukocytopenia, aplastic anemia and thrombocytopenia, etc.

Clinically, the patients with significant WBC decrease are mostly administered G-CSF, rhG-CSF injection (G-CSF) has more positive treatment effect, but expensive and efficacy is short-term, so it is not suitable for long-term use, the common complications include: fever, rash, muscle and joint pain and other adverse reactions. In which, muscle and joint pain often make the patients difficult to tolerate, seriously affects life quality of patients with malignant tumor during the treatment. At the same time, repeated injection of G-CSF can also cause dysfunction of bone marrow hematopoietic system, promote and induce myelodysplastic syndrome and acute myeloid leukemia, etc. If chemotherapy causes damage to bone marrow hematopoietic stem cell and lack of raw materials to general blood cells, or causes bone marrow reserve capacity of elderly patients is low, it is difficult to generate granulocytes if solely relying on G-CSF. In contrast, oral Leucocyte increasing drug has small adverse reactions, can be used for longer time and can improve the continuity and stability of treatment. However, the effect of WBC increasing is often not obvious if solely using oral drug, the combination of G-CSF and peroal Leucocyte increasing drug can make up with each other to get better effect. Therefore, clinically, it is more to combine injection with oral drugs to treat leucopenia or deficiency after chemotherapy. However, there is no uniform standard on how to combine these drugs, different hospitals have their own combination habits and cannot be unified. Some patients go to doctors of different hospitals in different stages of chemotherapy, but the prescriptions are various and different, even in order to treat the same degree of leucopenia or deficiency. The patients inadvertently become ‘drug jar’ and ‘experimental product’, and such unsystematic phenomenon seriously affected normative treatment and increased the probability of complications. Therefore, it is necessary to regulate drug combination of these patients.

The peroal Leucocyte increasing drugs involved in this study are commonly used in clinic, the main components and the mechanism to enhance WBC are different. In which, Leucogen and Berbamine are Western drugs, the main component of Leucogen is 2-(α- phenyl -α- ethoxycarbonyl - methyl) thiazolidine-4-carboxylic acid, and the main component of Berbamine is berbamine hydrochloride, both of them can increase WBC number through enhancing the hematopoietic function of bone marrow. The other three are traditional Chinese drugs, main effect of Qijiao Shengbai Capsule is to benefit qi and nourish blood, Wei Xuening has the efficacy of benefit and circulate blood and eliminate pathogenic heat from the blood, and the main effect of Compound Zaofan Pill is to warm the kidney and care bone marrow, supplement qi and nourish yin, enrich and stop blood, these three drugs can increase WBC number through benefit qi and nourish blood. The above five drugs can be used to treat chemotherapy-related leucopenia, and have been clinically proven better results (Tu et al., 2010; Wang et al., 2011; Wu et al., 2009; Xu et al.,2002; Zhang et al., 2008), however, at present, still lacks of contrastive study on speed and effect of above drugs, so the clinical use of these drugs lacks of proper guidance.

This study attempts to compare the five drugs’ effect through animal experiments. CTX is one of commonly used chemotherapy drugs in clinic and CTX injection in abdominal cavity of mice is chemotherapy-related leucopenia model preparation method commonly used in the clinic. This study makes rhG-CSF as treatment group positive control, it can increase total WBC number through enhancing the number of neutrophils, so there is increase of percentage of neutrophils and lymphocyte percentage is decreased relatively. Generally, chemotherapy drugs have inhibitory effect to blood three lines. This experiment proves that, after injecting CTX in abdominal cavity, the WBC, RBC, platelet and Hb concentration of mice are decreased in varying degrees. The study proves that the three-line value of model group is

significantly lower than what of normal control group. In all drugs, rhG-CSF has the strongest WBC increasing role, and in oral drugs, the WBC increasing effect of Leucogen is more prominent, significantly better than what of Qijiao Shengbai Capsule, Compound Zaofan Pill and Wei Xue Ning. RBC increasing effect of Compound Zaofan Pill, Berbamine tablet and Leucogen is better than what of Qijiao Shengbai Capsule. In oral drug treatment groups, HB increasing ability of Berbamine tablet and Leucogen is significantly better than what of Qijiao Shengbai Capsule, Wei Xue Ning and Compound Zaofan Pill, among them, the effect of Leucogen is the most prominent, and better than what of normal control group. PLT increasing effect of Compound Zaofan Pill, Berbamine tablet and Leucogen is significantly better than what of Qijiao Shengbai Capsule. We can know that part of Leucocyte increasing drugs has certain stimulating effect to other two-line hematopoietic function. Some papers reported (Wang et al.,2011) that Wei Xue Ning can increase the model's blood three-line level, inconsistent with the results of this paper, what may be related to differences in experimental conditions. This study shows that compared with other treatment groups, the lung index of Berbamine was significantly higher than what of Qijiao Shengbai Capsule, Wei Xue Ning, Compound Zaofan Pill and Leucogen, the liver index of Leucogen is significantly higher than that of Qijiao Shengbai Capsule. Berbamine and Leucogen respectively caused significant increase of lung and liver indexes, may be accompanied by related organ damage, such as: corresponding organ enlargement, at the same time, the two drugs also increased thymus index, what indirectly shows that the two drugs' immunomodulatory ability is strong and have a certain effect to immune suppression caused by CTX. The spleen index of Qijiao Shengbai Capsule is significantly higher than what of Berbamine and Leucogen, what shows that in oral drugs, the immunomodulatory ability of Qijiao Shengbai Capsule may be stronger. The spleen index of Qijiao Shengbai Capsule is significantly higher than what of Berbamine and Leucogen, what shows that in oral drugs, the immunomodulatory ability of Qijiao Shengbai Capsule may be stronger.

This study hopes to update the knowledge of clinicians to these drugs through experimental study, to help more standardized and flexible use in the clinic. However, the differences between people and animals may lead to different reactions to drugs, so the results still need further clinical study to validate.

Ethical approval

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The Committee on the Ethics of Animal Experiments of Zhejiang Cancer Hospital was secured for our research reported. Zhejiang Cancer Institutional Animal Care and Use Committee (IACUC) specifically approved this study. All authors abided the related rules of Committee on the Ethics of Animal Experiments of Zhejiang Cancer Hospital when this study began. All surgery was performed under sodium pentobarbital anesthesia, and all efforts were made to minimize suffering.

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